Understanding human disease via randomized mice

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Is epidemiology necessary?

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Outline

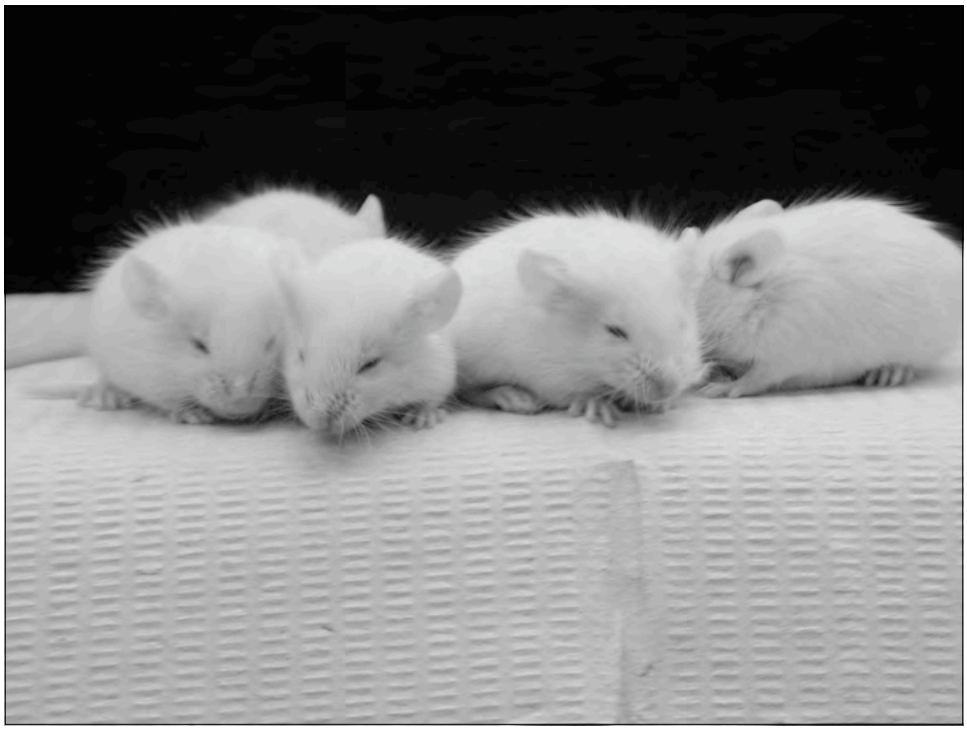
- Stuff that may be relevant to you.
- Stuff that is likely irrelevant, but hopefully will entertain you.

4

Goal

- Identify genes that contribute to common human diseases.

5



Why genetics?

- Phenotype → mechanism
- Need not know anything in advance.
- Genes may not be an important cause, but they can lead to
  - Disease etiology (e.g., pathways)
  - Possible drug targets

7

Approaches

- Model organisms (e.g. mouse or rat)
  - Mutagenesis
  - Experimental crosses
  - Association mapping
- Linkage analysis in human pedigrees
  - A few large pedigrees
  - Many small families (e.g., sibling pairs)
- Association analysis in human populations
  - Isolated populations vs. outbred populations
  - Whole genome vs. candidate genes/regions

8

Why mice?

Advantages	Disadvantages
+ Small and cheap	– Is the model really at all like the corresponding human disease?
+ Inbred lines	– Still not as small (or as fast at breeding) as a fly.
+ Simpler genetic architecture	
+ Controlled environment	
+ Large, controlled crosses	
+ Experimental interventions	
+ Knock-outs and knock-ins	

9

The mouse as a model

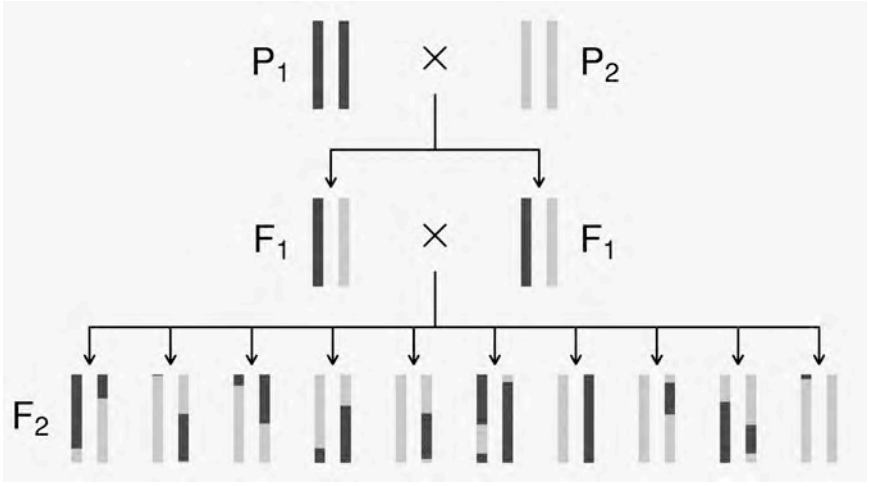
- Same genes?
  - The genes involved in a phenotype in the mouse may also be involved in similar phenotypes in the human.
- Similar complexity?
  - The complexity of the etiology underlying a mouse phenotype provides some indication of the complexity of similar human phenotypes.
- Transfer of statistical methods.
  - The statistical methods developed for gene mapping in the mouse serve as a basis for similar methods applicable in direct human studies.

10

C57BL/6

11

The intercross



12

Opportunities for improvement

- Each individual is unique.
  - Must genotype each mouse.
  - Unable to obtain multiple invasive phenotypes (e.g., in multiple environmental conditions) on the same genotype.
- Relatively low mapping precision.

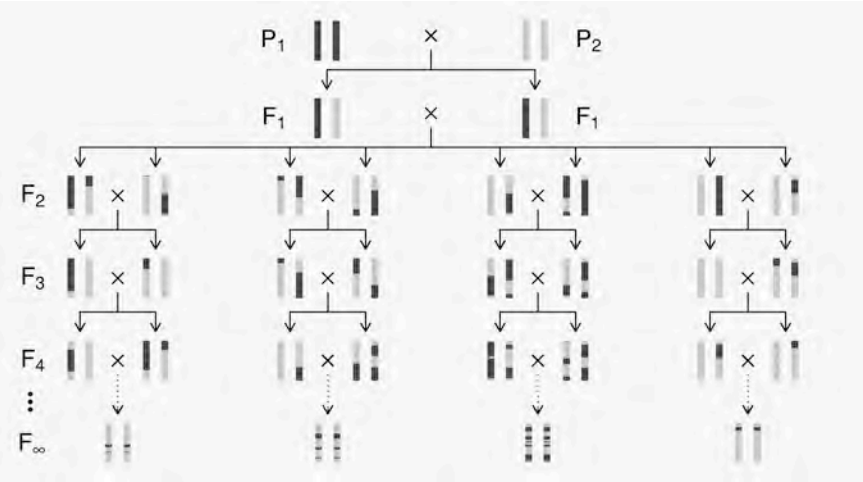
→ Design a set of inbred mouse strains.

  - Genotype once.
  - Study multiple phenotypes on the same genotype.

13

Recombinant inbred lines

(by sibling mating)



14

RI lines

Advantages

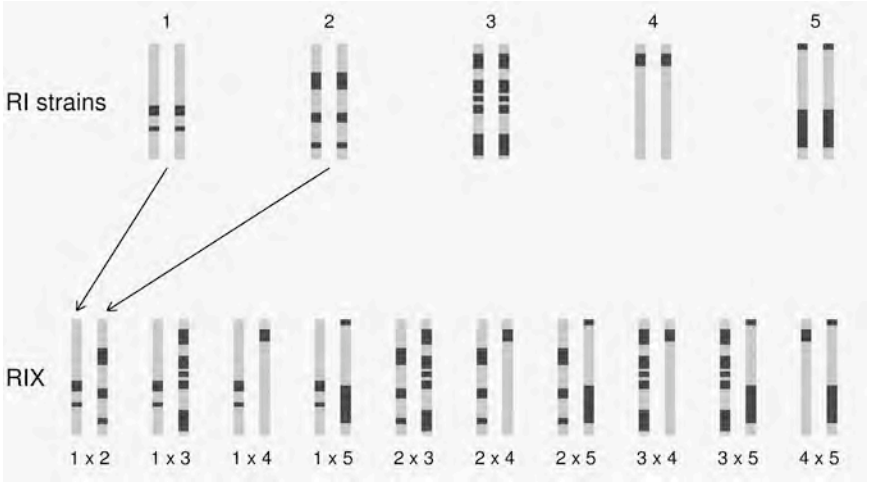
+ Each strain is a eternal resource.
  + Only need to genotype once.
  + Reduce individual variation by phenotyping multiple individuals from each strain.
  + Study multiple phenotypes on the same genotype.
+ Greater mapping precision.
  + More dense breakpoints on the RI chromosomes.

Disadvantages

– Time and expense.
– Available panels are generally too small (10-30 lines).
– Can learn only about 2 particular alleles.
– All individuals homozygous.

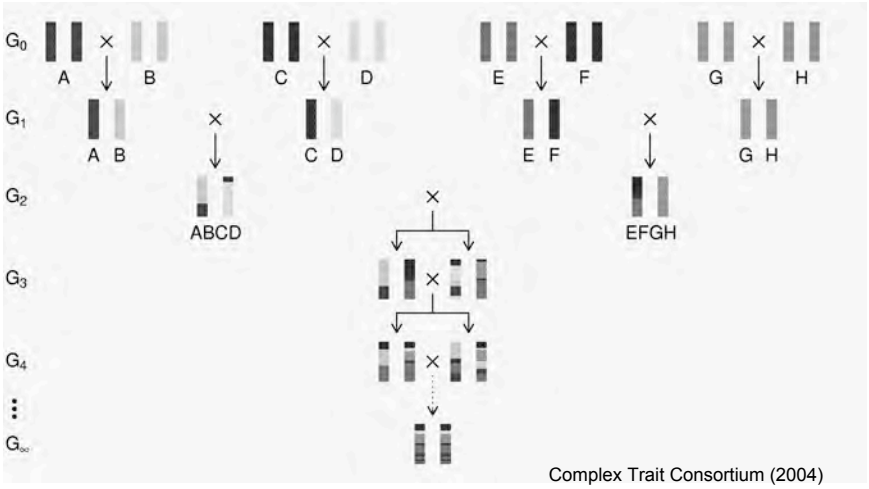
15

The RIX design



16

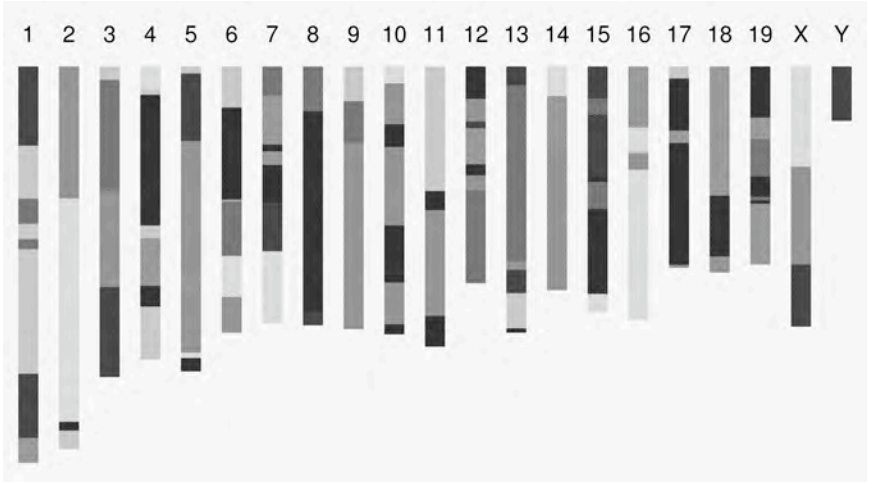
The Collaborative Cross



Complex Trait Consortium (2004)
Nat Genet 36:1133-1137

17

Genome of an 8-way RI



18

The Collaborative Cross

Advantages

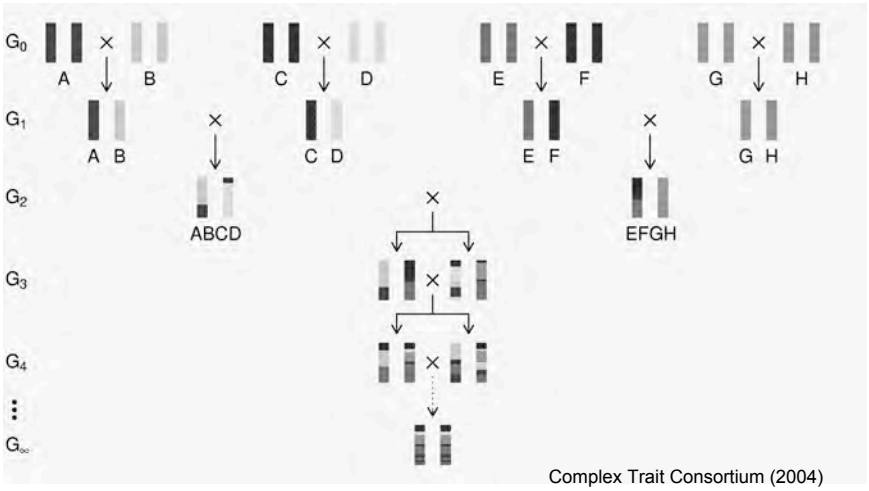
+ Great mapping precision.
+ Eternal resource.
  + Genotype only once.
  + Study multiple invasive phenotypes on the same genotype.

Barriers

- Advantages not widely appreciated.
  - Ask one question at a time, or Ask many questions at once?
- Time.
- Expense.
- Requires large-scale collaboration.

19

The Collaborative Cross



Complex Trait Consortium (2004)
Nat Genet 36:1133-1137

20

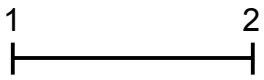
The goal

(for the rest of this talk)

- Characterize the breakpoint process along a chromosome in 8-way RILs.
  - Understand the two-point haplotype probabilities.
  - Study the clustering of the breakpoints, as a function of crossover interference in meiosis.

21

2 points in an RIL



- r = recombination fraction = probability of a recombination in the interval in a random meiotic product.
- R = analogous thing for the RIL = probability of different alleles at the two loci on a random RIL chromosome.

22

Haldane & Waddington 1931

INBREEDING AND LINKAGE*

J. B. S. HALDANE AND C. H. WADDINGTON
John Innes Horticultural Institution, London, England

Received August 9, 1930

Genetics 16:357-374

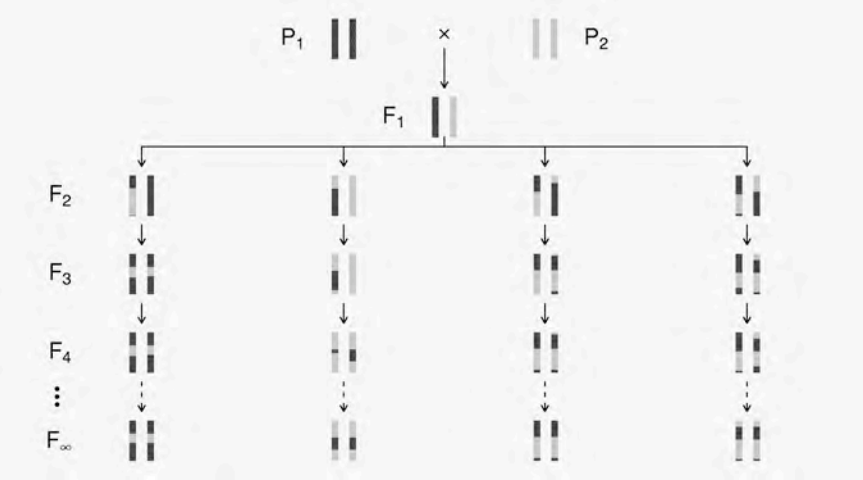
TABLE OF CONTENTS

When a heterozygous population is self-fertilized or inbred the ultimate result (apart from effects of mutation) is complete homozygosis. The final proportions of the various genotypes are usually independent of the system of inbreeding adopted, although, as JENNINGS (1916) and others have shown, the speed at which equilibrium is approached is greater in the case of self-fertilization than of brother-sister mating, and so on.

23

Recombinant inbred lines

(by selfing)



24

Markov chain

- Sequence of random variables $\{X_0, X_1, X_2, \ldots\}$ satisfying
  $$\Pr(X_{n+1} \mid X_0, X_1, \ldots, X_n) = \Pr(X_{n+1} \mid X_n)$$
- Transition probabilities $P_{ij} = \Pr(X_{n+1}=j \mid X_n=i)$
- Here, X_n = "parental type" at generation n
- We are interested in absorption probabilities
  $$\Pr(X_n \to j \mid X_0)$$

Equations for selfing

C_n $AABB$ and $aabb$.
D_n $AAbb$ and $aaBB$.
E_n $AABb$, $AaBB$, $Aabb$, and $aaBb$.
F_n $AB.ab$.
G_n $Ab.aB$.

We assume $2C_n+2D_n+4E_n+F_n+G_n=2$, so that $C_1=D_1=E_1=G_1=0$, and $F_1=2$. Clearly $E_\infty=F_\infty=G_\infty=0$, and D_∞ is the final proportion of crossover zygotes. Then considering the results of selfing each generation, we have:

$$\left.\begin{aligned}
C_{n+1} &= C_n + \tfrac{1}{2}E_n + \tfrac{1}{4}(1-\beta-\delta+\beta\delta)F_n + \tfrac{1}{4}\beta\delta G_n \\
D_{n+1} &= D_n + \tfrac{1}{2}E_n + \tfrac{1}{4}\beta\delta F_n + \tfrac{1}{4}(1-\beta-\delta+\beta\delta)G_n \\
E_{n+1} &= \tfrac{1}{2}E_n + \tfrac{1}{4}(\beta+\delta-2\beta\delta)(F_n+G_n) \\
F_{n+1} &= \tfrac{1}{2}(1-\beta-\delta+\beta\delta)F_n + \tfrac{1}{2}\beta\delta G_n \\
G_{n+1} &= \tfrac{1}{2}\beta\delta F_n + \tfrac{1}{2}(1-\beta-\delta+\beta\delta)G_n
\end{aligned}\right\} \quad (1.1)$$

…or C_{n+1}, D_{n+1}, and F_{n+1}, G_{n+1},

d_n } (1.2)

…r all values of n.
$- 2x)d_n$

$= \dfrac{1-2x}{1+2x}$.

Put $y=D_\infty$ (the final proportion of crossover zygotes)

$\therefore\ C_\infty + D_\infty = 1,\ C_\infty - D_\infty = c_\infty\ \therefore\ y = \tfrac{1}{2}(1-c_\infty).$

$$\therefore\ y = \frac{2x}{1+2x}. \quad (1.3)$$

Absorption probabilities

Let $P_{ij} = Pr(X_{n+1} = j \mid X_n = i)$ where X_n = state at generation n.

Consider the case of absorption into the state AA|AA.

Let h_i = probability, starting at i, eventually absorbed into AA|AA.

Then $h_{AA|AA} = 1$ and $h_{AB|AB} = 0$.

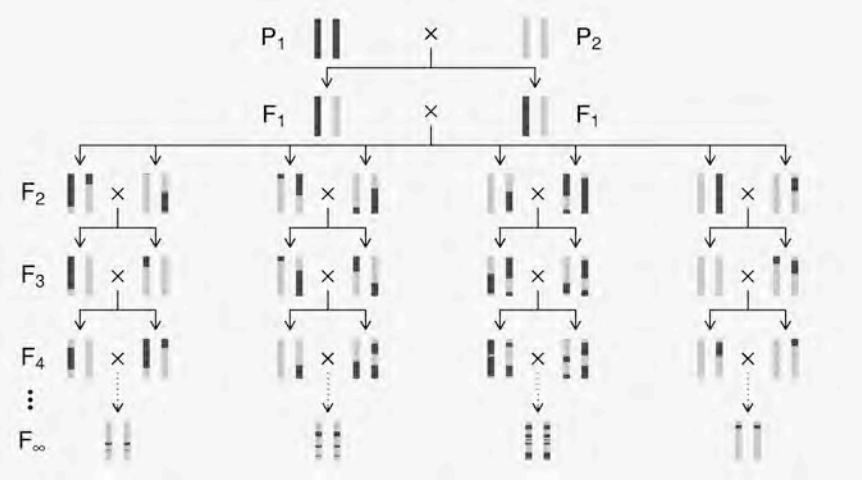
Condition on the first step: $h_i = \sum_k P_{ik} h_k$

For selfing, this gives a system of 3 linear equations.

27

Recombinant inbred lines

(by sibling mating)



28

Equations for sib-mating

Typical mating	Number of types	
$AABB\times AABB$	2	$C_{n+1}=C_n+H+\frac{1}{4}(\alpha^2+\gamma^2)L+\frac{1}{4}(\beta^2+\delta^2)N+\frac{1}{8}Q+\frac{1}{8}R+\frac{1}{8}(\alpha^2+\gamma^2)$ $U+\frac{1}{8}(\beta^2+\delta^2)V+\frac{1}{16}\alpha^2\gamma^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\beta^2\delta^2Y.$
$AAbb\times AAbb$	2	$D_{n+1}=D+I+\frac{1}{4}(\alpha^2+\gamma^2)M+\frac{1}{4}(\beta^2+\delta^2)P+\frac{1}{8}Q+\frac{1}{8}S+\frac{1}{8}(\beta^2+\delta^2)$ $U+\frac{1}{8}(\alpha^2+\gamma^2)V+\frac{1}{16}\beta^2\delta^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\alpha^2\gamma^2Y.$
$AABB\times aabb$	2	$E_{n+1}=\frac{1}{16}\alpha^2\gamma^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\beta^2\delta^2Y.$
$AAbb\times aaBB$	2	$F_{n+1}=\frac{1}{16}\beta^2\delta^2W+\frac{1}{16}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{16}\alpha^2\gamma^2Y.$
$AABB\times AAbb$	8	$G_{n+1}=\frac{1}{16}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}\alpha\beta\gamma\delta(W+2X+Y).$
$AABB\times AABb$	8	$H_{n+1}=\frac{1}{2}H+\frac{1}{16}($ $U+\frac{1}{16}($ $(\alpha\delta+\beta$
$AAbb\times AABb$	8	$I_{n+1}=\frac{1}{2}I+$ $U+\frac{1}{16}($ $(\alpha\delta+\beta$
$AABB\times Aabb$	8	$J_{n+1}=\frac{1}{16}($ $\beta\delta)(\alpha\delta$
$AAbb\times AaBB$	8	$K_{n+1}=\frac{1}{16}$ $\beta\delta)(\alpha\delta$
$AABB\times AB.ab$	4	$L_{n+1}=\frac{1}{4}(\alpha$ $\alpha^2\gamma^2W$
$AAbb\times Ab.aB$	4	$M_{n+1}=\frac{1}{4}($ $\beta^2\delta^2W+$

Typical mating	Number of types	
$AABB\times Ab.aB$	4	$N_{n+1}=\frac{1}{2}R+\frac{1}{8}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AAbb\times AB.ab$	4	$P_{n+1}=\frac{1}{2}S+\frac{1}{8}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{8}\alpha\beta\gamma\delta(W+2X+Y).$
$AABb\times AABb$	4	$Q_{n+1}=2G+\frac{1}{2}(H+I+J+K)+\frac{1}{4}(\alpha^2+\gamma^2)(L+M)+\frac{1}{4}(\beta^2+\delta^2)$ $(N+P)+\frac{1}{8}Q+\frac{1}{8}(R+S+T)+\frac{1}{16}(\alpha^2+\alpha\beta+\beta^2+\gamma^2+\gamma\delta+\delta^2)$ $(U+V)+\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times AaBB$	4	$R_{n+1}=\frac{1}{4}(\beta^2+\delta^2)L+\frac{1}{4}(\alpha^2+\gamma^2)N+\frac{1}{8}R+\frac{1}{8}(\beta+\delta)U+\frac{1}{8}(\alpha+\gamma)V+$ $\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times Aabb$	4	$S_{n+1}=\frac{1}{4}(\beta^2+\delta^2)M+\frac{1}{4}(\alpha^2+\gamma^2)P+\frac{1}{8}S+\frac{1}{8}(\alpha+\gamma)U+\frac{1}{8}(\beta+\delta)V+\frac{1}{16}$ $(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times aaBb$	4	$T_{n+1}=\frac{1}{8}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{16}(\alpha\delta+\beta\gamma)^2(W+Y)+\frac{1}{8}(\alpha\gamma+\beta\delta)^2X.$
$AABb\times AB.ab$	8	$U_{n+1}=\frac{1}{2}J+\frac{1}{4}(\alpha\beta+\gamma\delta)(L+N)+\frac{1}{8}(S+T)+\frac{1}{8}(\alpha+\gamma)U+\frac{1}{8}(\beta+\delta)$ $V+\frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)W+\frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X+\frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)Y.$
$AABb\times Ab.aB$	8	$V_{n+1}=\frac{1}{2}K+\frac{1}{4}(\alpha\beta+\gamma\delta)(M+P)+\frac{1}{8}(R+T)+\frac{1}{8}(\beta+\delta)U+\frac{1}{8}(\alpha+\gamma)$ $V+\frac{1}{8}\beta\delta(\beta\gamma+\alpha\delta)W+\frac{1}{8}(\alpha\gamma+\beta\delta)(\alpha\delta+\beta\gamma)X+\frac{1}{8}\alpha\gamma(\beta\gamma+\alpha\delta)Y.$
$AB.ab\times AB.ab$	1	$W_{n+1}=2(E+J)+\frac{1}{2}(\alpha^2+\gamma^2)L+\frac{1}{2}(\beta^2+\delta^2)N+\frac{1}{4}(S+T)+\frac{1}{4}(\alpha^2+\gamma^2)$ $U+\frac{1}{4}(\beta^2+\delta^2)V+\frac{1}{4}\alpha^2\gamma^2W+\frac{1}{4}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{4}\beta^2\delta^2Y.$
$AB.ab\times Ab.aB$	2	$X_{n+1}=\frac{1}{2}T+\frac{1}{2}(\alpha\beta+\gamma\delta)(U+V)+\frac{1}{2}\alpha\beta\gamma\delta(W+2X+Y).$
$Ab.aB\times Ab.aB$	1	$Y_{n+1}=2(F+K)+\frac{1}{2}(\alpha^2+\gamma^2)M+\frac{1}{2}(\beta^2+\delta^2)P+\frac{1}{2}(R+T)+\frac{1}{2}(\beta^2+$ $\delta^2)U+\frac{1}{2}(\alpha^2+\gamma^2)V+\frac{1}{2}\beta^2\delta^2W+\frac{1}{2}(\alpha^2\delta^2+\beta^2\gamma^2)X+\frac{1}{2}\alpha^2\gamma^2Y.$

29

Result for sib-mating

Omitting some rather tedious algebra, the solution of these equations is:

$$\zeta = \frac{q}{2-3q}, \quad \theta = \frac{2q}{2-3q}, \quad \kappa = \frac{1}{2-3q},$$

$$\lambda = \frac{1-2q}{2-3q}, \quad \mu = \frac{1-2q}{2-3q}, \quad \nu = \frac{2q}{2-3q}$$

as may easily be verified.

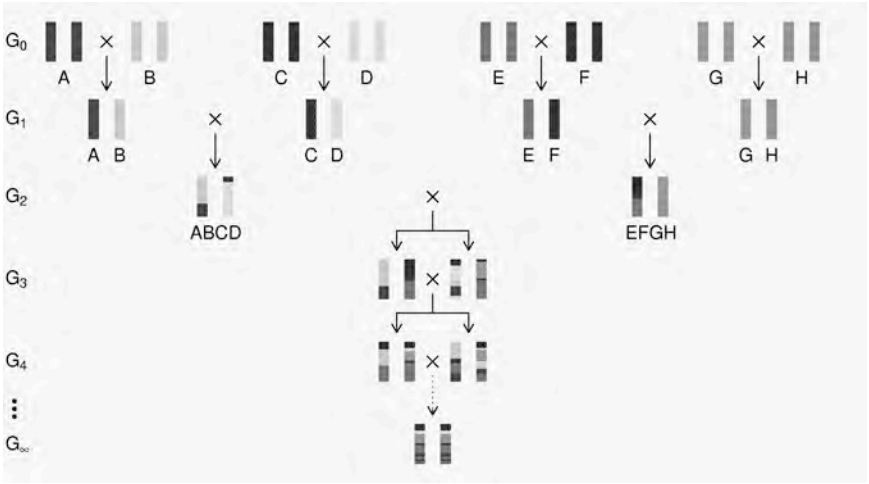
$$\therefore\ c_\infty = c_n + 2e_n + \frac{1}{1+6x}[(1-2x)(d_n + 2f_n + 2j_n + \tfrac{1}{2}k_n) + 2g_n + 4x(h_n + i_n)] \qquad (3.4)$$

and $y=\frac{1}{2}(1-c_\infty)$.

In the case considered, $d_0=1, \therefore c_\infty=\zeta d_0=1-2x/1+6x$. Hence the proportion of crossover zygotes, $y=4x/1+6x$ (3.5).

30

The "Collaborative Cross"



31

8-way RILs

Autosomes

$Pr(G_1 = i) = 1/8$

$Pr(G_2 = j \mid G_1 = i) = r / (1+6r)$ for $i \neq j$

$Pr(G_2 \neq G_1) = 7r / (1+6r)$

X chromosome

$Pr(G_1=A) = Pr(G_1=B) = Pr(G_1=E) = Pr(G_1=F) = 1/6$

$Pr(G_1=C) = 1/3$

$Pr(G_2=B \mid G_1=A) = r / (1+4r)$

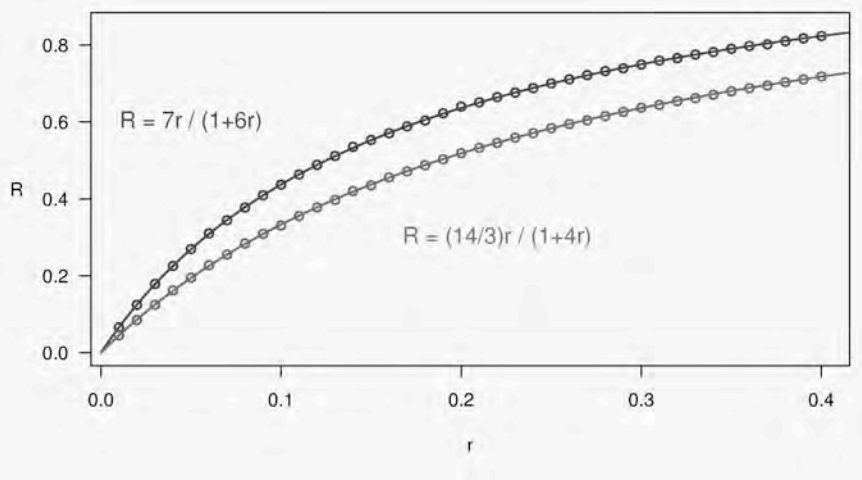
$Pr(G_2=C \mid G_1=A) = 2r / (1+4r)$

$Pr(G_2=A \mid G_1=C) = r / (1+4r)$

$Pr(G_2 \neq G_1) = (14/3)\, r / (1+4r)$

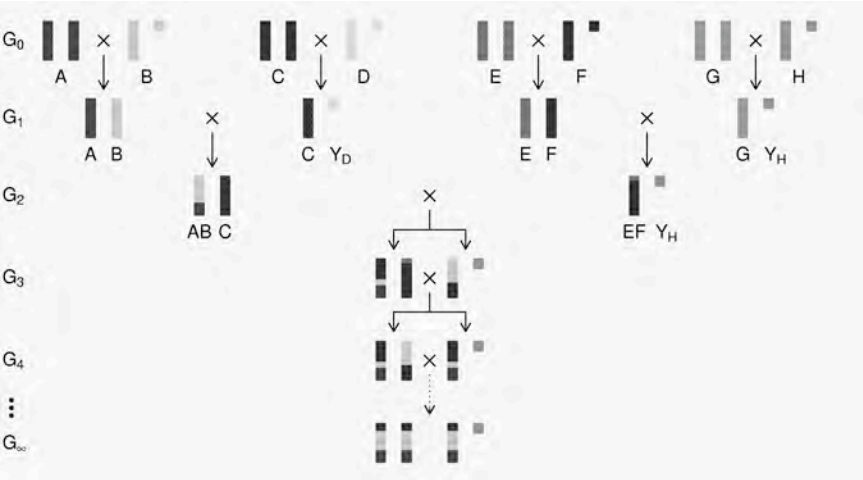
32

Computer simulations



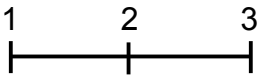
33

The X chromosome



34

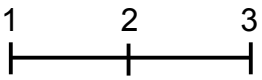
3-point coincidence



- r_{ij} = recombination fraction for interval i,j;
  assume $r_{12} = r_{23} = r$
- Coincidence = c = Pr(double recombinant) / r^2
  = Pr(rec'n in 23 | rec'n in 12) / Pr(rec'n in 23)
- No interference → = 1
  Positive interference → < 1
  Negative interference → > 1
- Generally c is a function of r.

35

3-points in 2-way RILs

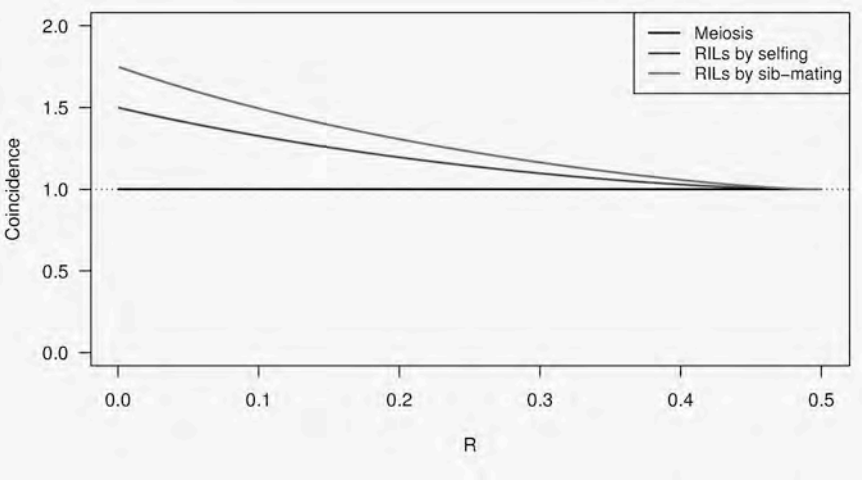


- $r_{13} = 2\ r\ (1 - c\ r)$
- $R = f(r)$; $R_{13} = f(r_{13})$
- Pr(double recombinant in RIL) = $\{ R + R - R_{13} \} / 2$
- Coincidence (in 2-way RIL) = $\{ 2\ R - R_{13} \} / \{ 2\ R^2 \}$

36

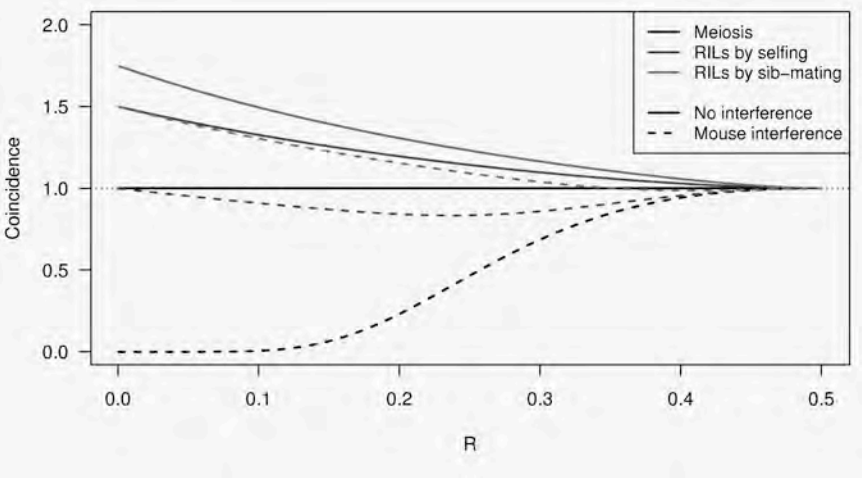
Coincidence

No interference



37

Coincidence



38

Why the clustering of breakpoints?

- The really close breakpoints occur in different generations.
- Breakpoints in later generations can occur only in regions that are not yet fixed.
- The regions of heterozygosity are, of course, surrounded by breakpoints.

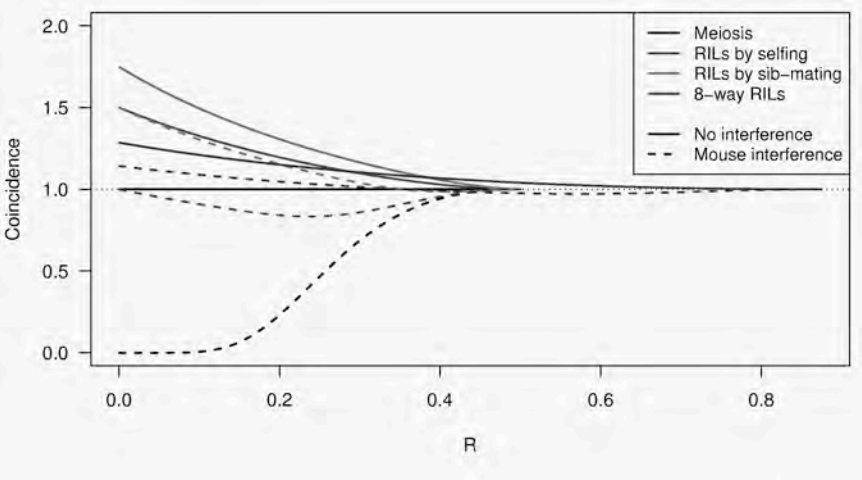
39

Coincidence in 8-way RILs

- The trick that allowed us to get the coincidence for 2-way RILs doesn't work for 8-way RILs.
- It's sufficient to consider 4-way RILs.
- Calculations for 3 points in 4-way RILs is still astoundingly complex.
  - 2 points in 2-way RILs by sib-mating:
    55 parental types → 22 states by symmetry
  - 3 points in 4-way RILs by sib-mating:
    2,164,240 parental types → 137,488 states
- Even counting the states was difficult.

40

Coincidence



41

Summary

- Mice are useful for learning about human disease.
- The Collaborative Cross could provide "one-stop shopping" for gene mapping in the mouse.
- Use of such 8-way RILs requires an understanding of the breakpoint process.
- We've extended Haldane & Waddington's results to the case of 8-way RILs: $R = 7\,r / (1 + 6\,r)$.
- We've shown clustering of breakpoints in RILs by sib-mating, even in the presence of strong crossover interference.
- Broman KW (2005) The genomes of recombinant inbred lines. *Genetics* 169:1133-1146

42